

sensitivity for cyprazine. After an appropriate warm-up time, with the range set at  $10^2$  and the sensitivity set at 16, a 21.72-ng ( $4 \mu\text{l}$  of a  $5.43 \mu\text{g}/\text{ml}$  solution) injection of cyprazine (equivalent to 20 ng of hydroxycyprazine) gives a peak height of 50–70 mm in slightly over 3 min.

**Preparation of a Standard Curve.** Standard solutions of cyprazine in toluene are prepared. To adjust for the difference in molecular weights of hydroxycyprazine (mol wt 209) and cyprazine (mol wt 227), concentrations of **2** are multiplied by 1.086 to yield the concentrations of **1** which are used as standards. A  $4\text{-}\mu\text{l}$  volume from each solution of cyprazine is injected into the gas chromatograph under conditions described above. A plot of the logarithm of the concentration of cyprazine ( $1.086 \times$  hydroxycyprazine) vs. the logarithm of the recorder response yields a straight-line correlation.

The response of the nitrogen-sensitive detector is highly dependent upon detector temperature and flow rates of carrier, hydrogen, and air, causing changes in response magnitude over a period of time; therefore the standard should be re-injected frequently.

#### RESULTS AND DISCUSSION

Known amounts of **2** were added to corn silage or grain prior to extraction to determine the efficiency of the procedure. Determinations of **2** from 50-g samples averaged 73% (range 62–88%, standard deviation  $\pm 7\%$ ) for added levels representing 0.01, 0.02, 0.05, 0.10, and 0.20  $\mu\text{g}/\text{g}$  (ppm).

Interfering plant background arising from untreated corn silage or grain is usually nonexistent in this procedure. However, a peak at the retention time of cyprazine corresponding to not more than an apparent 0.015  $\mu\text{g}$  of cyprazine/g corn is occasionally seen. A level of twice the natural background is arbitrarily chosen as significant, which results in a sensitivity of 0.03 ppm for this method. No hydroxycyprazine residues (*i.e.*, above 0.03 ppm, the sensitivity of the analytical procedure)

were found in any harvested field samples which had been treated with cyprazine.

The chlorination reaction is not a simple exchange but involves attack of a tautomeric amide form of **2** by a Vilsmeier-type complex of the phosphorus pentachloride and dimethyl formamide. The completeness of this reaction and workup is the yield-limiting step in the analytical procedure. From 75 to 85% conversion in this step is routine in the range of 1–50  $\mu\text{g}$  of **2**.

There appears to be no portion of this method which would preclude its application to any other 2-hydroxy-4,6-dialkylamino-*s*-triazines, save a slight adjustment in the elution volumes for the final alumina column.

#### LITERATURE CITED

- Chen, J.-Y. T., *J. Ass. Offic. Anal. Chem.* **50**, 595 (1967).  
 Fishbein, L., *Chromatogr. Rev.* **12**, 167 (1970).  
 Flint, G. T., Aue, W. A., *J. Chromatogr.* **52**, 487 (1970).  
 Harris, C. I., *J. AGR. FOOD CHEM.* **15**, 157 (1967).  
 Lamoureux, G. L., Shimabukuro, R. H., Swanson, H. R., Frear, D. S., *J. AGR. FOOD CHEM.* **18**, 81 (1970).  
 Montgomery, M. L., Botsford, D. L., Freed, V. H., *J. AGR. FOOD CHEM.* **17**, 1241 (1969).  
 Montgomery, M. L., Freed, V. H., *J. AGR. FOOD CHEM.* **12**, 11 (1964).  
 Riden, J. R., Asbell, W. J., Midwest Regional Meeting of the American Chemical Society, Kansas City, Missouri, 1969.  
 Riden, J. R., Asbell, W. J., Schroeder, R. S., Abstracts of Papers, 160th National Meeting, of the American Chemical Society, Chicago, Illinois, 1970, PEST 7.

Robert S. Schroeder\*  
 Natu R. Patel  
 Loren W. Hedrich  
 W. Carter Doyle  
 Joseph R. Riden  
 Lee V. Phillips

Gulf Research and Development Company  
 9009 W. 67th Street  
 Merriam, Kansas 66202

Received for review May 22, 1972. Accepted July 12, 1972.

## Anomalous Effects of Humidity Control

Oxidation of methyl linoleate supported on microcrystalline cellulose showed anomalous effects when humidified to  $A_w = 0.62$ . The highly catalytic

effect was shown to be due to diffusion of dinitrogen trioxide formed in the saturated  $\text{NaNO}_2$  solution through the vapor space into the samples.

Saturated salt solutions have been a convenient and reliable means of producing static constant relative humidity (water activity) conditions for study of experimental systems (Carr and Harris, 1949; Rockland, 1960). In most cases no interaction is expected between the solution and the system suspended in the vapor space above the solution unless some halide salt is used, such as KBr, which can give off bromine vapors.

In studying the phenomenon of moisture sorption hysteresis and its effect on metal-catalyzed lipid oxidation, we expected that a system mixed dry (cellulose powder–glycerol–methyl linoleate) and humidified to a specific water activity would oxidize at a much slower rate than that of systems which were mixed directly with all ingredients including water to that specific activity (Labuza *et al.*, 1972). This is due to

the fact that sorption hysteresis occurs with the direct mixed system having a higher equilibrium moisture content and thus a lower viscosity aqueous phase for transport of catalysts. This phenomenon occurred at all water activities studied [ $0.52 \text{ Mg}(\text{NO}_3)_2$ ,  $0.68 \text{ SrCl}_2$ ,  $0.75 \text{ NaCl}$ ,  $0.84 \text{ K}_2\text{CrO}_4$ , and  $0.89 \text{ KNO}_3$ ] except at 0.62. The latter water activity was obtained by using a saturated  $\text{NaNO}_2$  solution and holding under vacuum for 24 hr. As shown in Figure 1, the humidified sample at 0.62 (DH) oxidized faster than any other sample and did not fit in the above hypothesis.

In order to determine if it was some factor contributed by the salt solution, the experiment was repeated but all directly mixed samples with water were held also in the desiccators simultaneously during rehumidification of the other sample set (24 hr). Oxygen uptake by the Warburg manometric

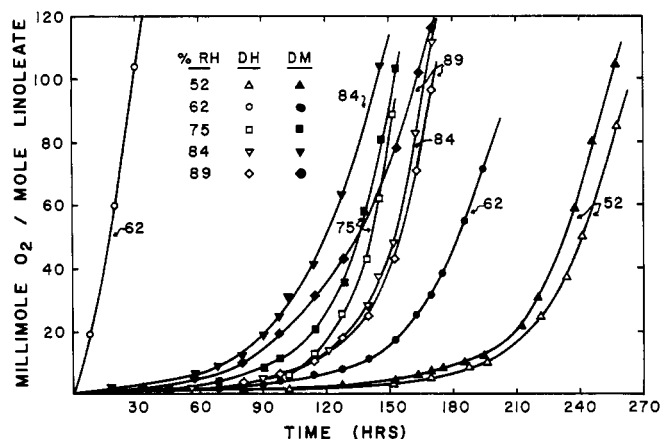


Figure 1. Oxygen absorption by linoleate model systems at various relative humidities (DH-dried and rehumidified; DM-direct mixed)

technique was measured again and, as shown in Figure 2, both systems at 0.62 oxidized most rapidly, while the other water activities gave results similar to the first test.

These results indicated that the saturated  $\text{NaNO}_2$  solution decomposed and produced something that transferred into the samples. (Proper precautions were taken during the humidification to prevent bubbling over of the solution which would contaminate the samples.) The samples were analyzed for  $\text{NO}_2^-$  and  $\text{NO}_3^-$  and the results are shown in Table I. As seen, the cellulose powder contains no nitrite initially but, when humidified at 0.62, both  $\text{NO}_2^-$  and  $\text{NO}_3^-$  content increases. The directly mixed sample (DM) shows very similar results, an increase in  $\text{NO}_3^-$  and appearance of  $\text{NO}_2^-$  in the sample if put in the desiccator. The rehumidified dry samples show the presence of a corresponding amount of these compounds. A freshly prepared  $\text{NaNO}_2$  solution produced lower quantities of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in the samples. In no case did nitrite or nitrate content increase in systems humidified at other conditions, including saturated nitrate salts.

To explain the phenomenon, it is proposed that since the solution is slightly acid (pH 5.8 from distilled water used), nitrous acid can be formed by  $2\text{H}^+ + 2\text{NO}_2^- \rightleftharpoons \text{HNO}_2 \rightleftharpoons \text{H}_2\text{O} + \text{N}_2\text{O}_3 \uparrow$  which, as shown, decomposes to  $\text{N}_2\text{O}_3$  and escapes from the solution. This  $\text{N}_2\text{O}_3$  will readsorb on the cellulose-based samples; the reverse reaction occurs. The older solution probably absorbs  $\text{CO}_2$  from the air, making it more acid. The reason for the catalytic effect in the presence of  $\text{NO}_2^-$  can be due to reductive activation in that the nitrite quickly converts the trace metals (in all systems 100 ppm of added cobalt) into the reduced state, where they are more active as a catalyst (Labuza, 1971). It is also possible that the nitrite salts could decompose the hydroperoxides

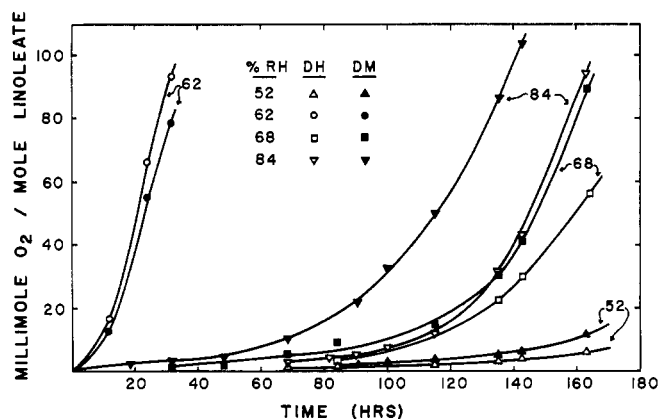


Figure 2. Oxygen absorption by linoleate model systems at various relative humidities (DH-dried and rehumidified; DM-direct mixed and held in desiccator at given % relative humidity)

Table I. Nitrite and Nitrate Content (ppm)

		Dry cellulose powder	Humidified cellulose powder	Humidified dry mix	Direct mix (as is)	Direct mix humidified
Old solution	$\text{NO}_2^-$	0	1.71	7.62	0	9.80
	$\text{NO}_3^-$	2.5	3.40	1.00	3.40	1.60
Fresh solution	$\text{NO}_2^-$	0	1.45	3.04	0	4.10
	$\text{NO}_3^-$	2.5	3.40	1.00	3.40	0.91

formed. The true mechanism has not been investigated. This study points out that one must be extremely careful in choosing a humidity-controlling system.

#### ACKNOWLEDGMENT

Paper No. 7909, Scientific Journal Series, Minnesota Agricultural Experiment Station.

#### LITERATURE CITED

- Carr, D. S., Harris, B. L., *Ind. Eng. Chem.* **41**, 2014 (1949).  
 Labuza, T. P., McNally, L., Gallagher, D., Hawkes, J., Hurtado, F., *J. Food Sci.* **37**, 154 (1972).  
 Labuza, T. P., *Crit. Rev. Food Technol.* **2**, 355 (1971).  
 Rockland, L. B., *Anal. Chem.* **32**, 1375 (1960).

Hung-En Chou  
 Theodore P. Labuza\*

Department of Food Science and Nutrition  
 University of Minnesota  
 St. Paul, Minnesota 55101

Received for review June 1, 1972. Accepted August 21, 1972.